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Original article

Presentation of hemolytic and hemorrhagic rangeliosis in *Cerdocyon thous*

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ABSTRACT

Rangeliosis, caused by protozoan *Rangelia vitalii*, is transmitted by the tick *Amblyomma aureolatum*. The disease is characterized by hemolytic and hemorrhagic disorder and has been described in dogs and other wild canids. The aim of this study was to compare clinicopathological findings and laboratory results of a *Rangelia* infection in a crab-eating fox (*Cerdocyon thous*) with those of canine rangeliosis. The zoo of Universidade de Caxias do Sul, received a crab-eating fox with marked jaundice in mucous membranes, dark-colored stools and neurological signs. The animal underwent an ear tip smear examination and blood collection for complete blood counts, serum biochemistry and PCR. Free-living and intraerythrocytic pyriform structures consistent with *R. vitalii* were found in the blood smear of the ear tip. The erythrogram revealed normocytic normochromic anemia, moderate macrocytosis, polychromasia and metarubricytosis. The leukogram revealed leukocytosis with neutrophilia and monocytosis, as well as severe thrombocytopenia. Serum biochemistry showed hypoproteinemia, hypoalbuminemia and elevated levels of urea and creatinine. The treatment was performed with imidocarb hydrochloride and dexamethasone, however 24 h after initiation of treatment the animal died. Macroscopic examination revealed jaundice, subcutaneous edema, enlarged superficial lymph nodes, splenomegaly, and hemorrhage of internal organs. Histological sections of the cerebellum, lung, pancreas, intestine and heart were consistent with *R. vitalii* infection of the vascular endothelium. Pathological and hematological findings were similar to those found in infected dogs, with clinical presentation characterized by hemolytic anemia and hemorrhage. The description of this case showed that *C. thous* does not only serve as reservoir of *R. vitalii* but may also develop disease.

1. Introduction

Rangeliosis is a disease caused by the protozoan *Rangelia vitalii*; the vector for this protozoan is the tick *Amblyomma aureolatum* (Soares et al., 2018). To date, the protozoan has been described in dogs and other wild canines (França et al., 2010; Soares et al., 2014; Fredo et al., 2015; Quadros et al., 2015; Soares et al., 2015). The diagnosis is achieved by observing parasites in the peripheral blood that can be located both free and inside red blood cells and white blood cells. In addition, the diagnosis can be made via histological visualization in endothelial cells of several tissues and can be confirmed by PCR (Fighera et al., 2010; Soares et al., 2011; Lemos et al., 2012; França et al., 2014; Soares et al., 2015).

The clinicopathological signs observed in dogs with rangeliosis include anemia, jaundice, apathy, fever, hepatomegaly, splenomegaly, lymphadenopathy, hematochezia, hematemesis, petechiae, skin suffusions and bleeding on the external surface of the ears and nostrils (Loretti and Barros, 2005; Fighera et al., 2010; França et al., 2010; Da Silva et al., 2011).

The pathogenesis of rangeliosis in dogs has not yet been fully elucidated, however, in recent years various research groups have described the laboratory findings and clinical presentations. In wild canids, the protozoan was described in the species *C. thous* and *Lycalopex gymnocercus*, but without the hemorrhagic and hemolytic presentation characteristic of rangeliosis (Soares et al., 2014; Fredo et al., 2015; Quadros et al., 2015). In this study, we report a case of rangeliosis in a crab-eating fox (*C. thous*) that presented with clinical, laboratory and pathological signs, with confirmation of the protozoan by ear-tip blood smear, histopathology and by PCR.

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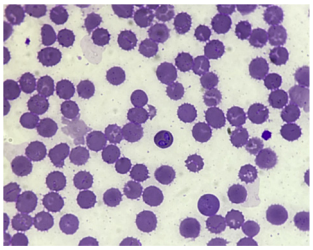

Fig. 1. Ear tip blood smear from the *Cerdocyon thous*, showing pyriform inclusions inside erythrocytes, consistent with *R. vitalii*. Romanowsky stain, 100× magnification.

2. Case report

A male wild crab-eating fox, approximately 2 years old, weighing 2.5 kg, was sent to the zoological park of the University of Caxias do Sul after having been captured in the city of Garibaldi (29°17′20″S 51°33′51″W), Rio Grande do Sul, Brazil. On clinical examination, the animal manifested motor incoordination, disorientation, intense jaundice in the oral and ocular mucosa, and feces with blackish coloration. In addition, ticks were found, identified as *A. aureolatum*.

Blood was collected from the jugular vein to perform hemogram and serum biochemistry, as well as from the ear tip for hemoparasite screening. The ear tip smear was Romanowsky-stained, revealing pyriform inclusions both within and free of erythrocytes that were consistent with merozoites of large piroplasms, probably *R. vitalii* (Fig. 1). The erythrogram revealed normocytic normochromic anemia. On morphological evaluation, there was moderate anisocytosis, polychromasia and metarubricytosis. The leukogram revealed leukocytosis with neutrophilia and monocytosis, as well as severe thrombocytopenia. Serum biochemistry showed hypoproteinemia, hypoalbuminemia and elevated levels of urea and creatinine (Table 1).

For molecular biological analysis, DNA was extracted from blood samples using a commercial silica-based kit (Simbios, Brazil). PCR reactions were performed using GoTaq® Green Master Mix (Promega, Brazil) with 10 pmol of two pairs of primers described by Spolidorio et al. (2009): BAB-33-57 (5-GCCAGTAGTCATATGCTTGTCTTAA-3), BAB-432-409 (5-TTCCTTAGATGT GGT AGC CGT TTC-3), BAB143-167 (5-CCGTGCTAATTGTAGGGCTAATACA-3) and BAB694-667 (5-GCTTGAAACACTCTARTTTTCTCAAAG-3). An aliquot of 2 µl of template DNA was added to a total volume of 25 µL in each reaction. Amplification was performed with an initial denaturation at 95 °C for 2 min, followed by 35 cycles of 60 s at 95 °C, 60 s at 55 °C and 90 s at 72 °C, with a final extension at 72 °C for 5 min. Then, the amplified segments were purified using a Gel Purification Kit (Ludwig Biotecnologia, Brazil), sequenced using ACTGene Análises Moleculares (Alvorada, Brazil) and the generated sequence was deposited in the GenBank as number MG967621. The analysis was performed with Lasergene software (DNASTAR), MEGA7 (Kumar et al., 2016) and the BLAST platform (accessed at < https://blast.ncbi.nlm.nih.gov/Blast.cgi >). A total of 99% identity was verified with six sequences of *R. vitalii*, deposited in the GenBank (*e-value = 0.0*), four from Rio de Janeiro (JN880429-JN880432) and two from Argentina (KF218605 and KF218606). The phylogenetic analysis is summarized in Fig. 2.

Table 1
Results of the hematological and biochemical analyses performed on blood samples from a *Cerdocyon thous*.

Parameters	*C. thous*	Reference values Gomes (2006)
Erythrocytes (×10^6/µL)	3.05	4.31–6.77
Hemoglobin (g/dL)	8.4	12.96–16.88
Packed cell volume (%)	27	38–49
VCM (fL)	88	68–95
CHCM (%)	31	31–38
Metarubricyte (/100 leukocytes)	15	
Leukocytes (/ µL)	18,000	8,100–13,900
Neutrophils (/µL)	14,220	5,758–10,387
Lymphocytes(/µL)	2,160	1,062–2,357
Monocytes (/µL)	1,620	0–354
Platelets (/µL)	15,000	268,000
Total Protein (g/dL)	4.75	5.47–7.09
Albumine (g/dL)	2.08	2.44–3.98
ALT (UI/L)	25	12–52
AST (UI/L)	22	19–54
Creatinine (mg/dL)	5.11	0.37–1.11
FA (UI/L)	105	232.43
Urea (mg/dL)	352.2	22.46–71.84

The animal died 24 h after initiation of treatment with imidocarb hydrochloride (5 mg/kg SC) and dexamethasone (0.25 mg/kg SC), and the body was sent for necropsy. On gross examination, there was intense generalized jaundice of the mucous membranes and cutaneous tissues (Fig. 3). In the subcutaneous tissue there was marked edema of limbs, and in the ventral region there was jaundice and enlargement of superficial lymph nodes. Splenomegaly was observed and the cut surface of the organ appeared pulpy. Focal extensive bleeding was identified in the pancreas. The intestinal mucosa presented a large quantity of petechiae. In the lumen there was a fair amount of dark content (melena). The liver was enlarged and jaundiced. The lungs were erythematous with pleural emphysema at the borders. Fragments of several organs were fixed in 10% formalin, processed by routine histological techniques and subjected to microscopic analysis.

Histology revealed discrete zoites forming parasitic cell vacuoles, consistent with *R. vitalii* in the vascular endothelium of the heart (Fig. 4), cerebellum, lymph nodes, lungs, small intestine and pancreas. In the brain, discrete inflammatory infiltrates of lymphocytes and multifocal perivascular macrophages were visualized. Lymph nodes contained moderate multifocal centrofollicular necrosis and some macrophages with erythrophagocytosis. In the spleen, we found moderate multifocal hemosiderosis. Analysis of the small intestine revealed multifocal areas of mucosal hemorrhage. In the lungs, there was marked multifocal hemorrhage in the alveoli. The liver showed mild extramedullary hematopoiesis. In the bone marrow there were discrete decreased myeloid lineages. There was slight hemorrhage in the submucosa of the urinary bladder.

3. Discussion

The disease occurs most frequently in the hottest times of the year in the southern region of Brazil, when there are more vectors in the environment, and animals from rural areas are more predisposed to develop the disease (Soares et al., 2014). The animal in question was captured in the summer and he was infested with ticks identified as *A. aureolatum*, corresponding to the only species involved in the transmission of *R. vitalii* in dogs (Fighera, 2007; Da Silva et al., 2011); it is an originally-occurring parasite in wild foxes (Labruna and Pereira, 2001; Guglielmone et al., 2003).

Soares et al. (2014), described a *C. thous* infected with *R. vitalii* that remained for at least eighty days without clinical or hematological manifestations. This animal had been referred for care due to a fracture. Fredo et al. (2015), reported a *C. thous* with a history of paralysis and myoclonus, and a *L. gymnocercus* that had been attacked by dogs and was referred for necropsy. The *C. thous* was also positive for distemper virus, justifying the presence of myoclonus. The diagnosis of rangeliosis was made in both animals through histopathological examination. Quadros et al. (2015) reported a case of rangeliosis in an *L. gymnocercus*. The animal was dehydrated, with pale mucosa, apathy, hypothermia and incoordination. In the case reported here, the animal presented apathy, jaundice, edema, blackened stools, hemorrhage and enlargement of lymphoid organs, clinical signs consistent with the description in domestic canines with the disease (Fighera et al., 2010). In addition, the animal presented with neurological alterations not

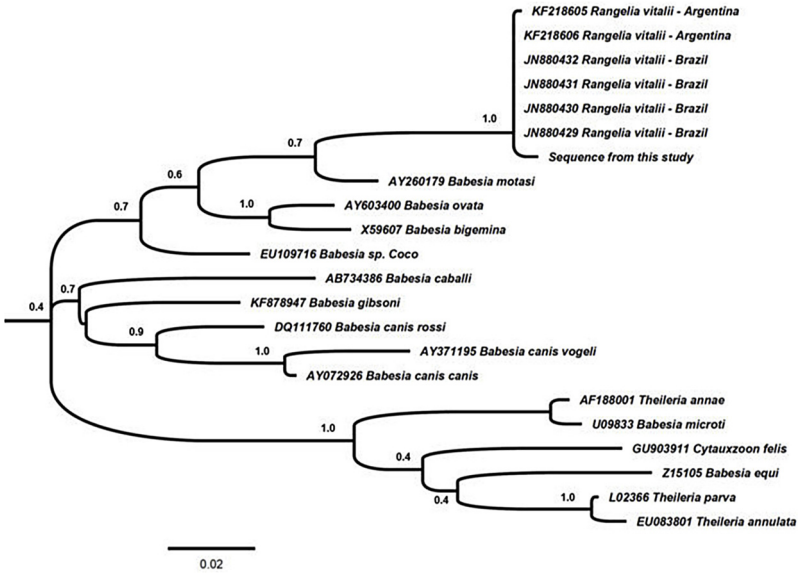

Fig. 2. Hemolytic and hemorrhagic presentation of rangeliosis in a *Cerdocyon thous*. Maximum likelihood phylogeny generated from 18S gene sequences of rRNA from *Rangelia vitalii* (JN880429, JN880430, JN880431, JN880432, KF218605, KF218606), *Babesia motasi* (AY260179.1), *B. ovata* (AY603400.1), *B. gibsoni* (KF878947.1), *B. vogeli* (AY371195.1), *B. canis* (AY072926.1), *B. rossi* (DQ111760.1), *Babesia* sp. Coco (EU109716.1), *B. bigemina* (X59607.1), *B. caballi* (AB734386.1), *B. microti* (U09833.1), *Theileria annae* (AF188001.1), *T. equi* (Z15105.1), *T. parva* (L02366.1), *T. annulata* (EU083801.1) and *Cytauxzoon felis* (GU903911.1). We used the Kimura-2-parameter replacement model with 6 gamma categories. The tree was designed for scale, with the length of branches measured from the number of substitutions per site. Confidence was measured using the bootstrap method inferred by 1000 repetitions.

Fig. 3. Pathological findings observed at necropsy of *Cerdocyon thous* with rangeliosis. A- oral cavity jaundice. B- splenomegaly and jaundice of organs of the abdominal cavity. C- mucosa small intestine with jaundice and petechiae. D- intestinal jaundice and dark lumen content.

frequently reported in dogs, probably secondary to encephalitis associated with the parasite.

Changes usually found in the erythrogram of animals infected by the protozoan are compatible with extravascular immune-mediated hemolytic anemia. In naturally and experimentally infected dogs, the observed anemia is usually macrocytic normochromic or normocytic normochromic. The morphological changes are anisocytosis, polychromasia, Howell-Jolly bodies, metarubricytosis, spherocytosis and erythrophagocytosis (Fighera et al., 2010; França et al., 2010, França et al., 2013). *C. thous* presented the same laboratory findings as those described for the disease in dogs. The anemia was probably caused by the destruction of erythrocytes in the mononuclear phagocytic system.

Leukocytosis is characterized by lymphocytosis, monocytosis and in some cases left-shift on white blood cell differential (Fighera, 2007; Fighera et al., 2010; França et al., 2010). In this case, the animal presented leukocytosis with neutrophilia and monocytosis, characterizing a chronic inflammatory response. The animal presented severe thrombocytopenia, a frequent hematological finding in canine rangeliosis (Fighera et al., 2010; França et al., 2010; Paim et al., 2012). This laboratory alteration confirmed the hemorrhagic findings observed at necropsy.

In rangeliosis, there is no specific change in biochemical tests, however performance of these tests may be indicated as part of the evaluation of general state (Fighera, 2007). Although there are no specific alterations in serum biochemistry during rangeliosis (Fighera, 2007; Paim et al., 2013; Soares et al., 2014; França et al., 2014), Costa et al. (2012) observed an increase in alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK) and bilirubin levels in the sera of experimentally infected dogs.

Serum biochemistry of infected *C. thous* showed hypoproteinemia, hypoalbuminemia, consistent with the findings of subcutaneous edema observed at necropsy. Hypoalbuminemia and hypoproteinemia were

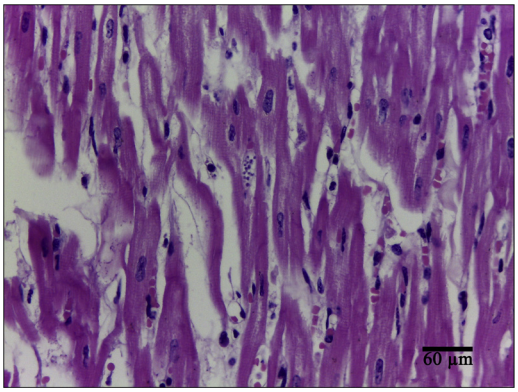

Fig. 4. Histological section of the heart of *Cerdocyon thous* with zoites of *R. vitalii* forming parasitic vacuoles in vascular endothelium. Hematoxylin and eosin stain. Bar = 60 µm.

reported in dogs experimentally infected with *R. vitalii*. These alterations were related to inflammation, because albumin is a negative acute phase protein its synthesis may decrease due to inflammatory process and vasculitis and other inflammatory conditions are common pathological findings in rangeliosis (Paim et al., 2013). The anorexia and hemorrhage describe in dogs infected with *R. vitalii* may be other causes of hypoalbuminemia (Stockham and Scott, 2002). Elevated urea levels are probably due to blood loss through the gastrointestinal tract. Also, extravascular haemolysis leads to increased serum levels of bilirubin in canine rangeliosis (França et al., 2014). Bilirubin pigments are nephrotoxic and may cause elevation in urea and creatinine.

Among the pathological findings, many of the lesions found have also been identified in canine rangeliosis. Mucosal jaundice is commonly observed in infected dogs. In this species it was described in 89%–100% of cases (Fighera et al., 2010; Fredo et al., 2015). The most frequently identified lesions in infected dogs are splenomegaly, hepatomegaly, hepatic discoloration, lymphadenopathy and intestinal hemorrhage, all of which were seen in the present case. Splenomegaly is described in all dogs with rangeliosis, aiding in the differential diagnosis of other diseases that present with jaundice (Fighera et al., 2010; Fredo et al., 2015). Lymph node enlargement was present in several cases, where it was reported in 77.1%–83% of dogs (Fighera et al., 2010; Fredo et al., 2015). Jaundice, splenomegaly and lymphadenopathy characterize extravascular hemolysis.

The development of subcutaneous edema in pelvic limbs was also observed in dogs, in 4% to 17.1% of necropsies, associated with protein reduction from blood loss (Fighera et al., 2010; Fredo et al., 2015). In the present study, hypoproteinemia was also responsible for the observation of edema in other anatomical locations, including ascites and hydrothorax, which in dogs were identified in 17.1% to 25.7% of cases, respectively. An important lesion in rangeliosis is the observation of intestinal hemorrhage with the observation of petechiae, melena or hematochezia. In the present report, marked hemorrhage corresponded to the formation of petechiae in the intestinal mucosa in association with melena. Pancreatic hemorrhage was identified in only 8.2% of dogs (Fredo et al., 2015).

In dogs, in numerous organs, parasitic structures consistent with *R. vitalii* have been visualized. Histology revealed zoites in the heart, lung, small intestine and pancreas; such organs are commonly affected by protozoa, sometimes at high concentrations in vascular endothelium (Fredo et al., 2015). Interstitial nephritis and mononuclear myocarditis associated with parasitic vacuoles of *R. vitalii* are characteristic in infected dogs (Fighera et al., 2010; Fredo et al., 2015), however, they were not observed in the present case. Discrete mononuclear perivascular inflammatory infiltrates in the brain have been described in 29.6% of infected dogs (Fighera et al., 2010). In lymphoid organs, the lesions of erythrophagocytosis, hemosiderosis and necrosis of lymphoid follicles are commonly seen in infected domestic canines (Fredo et al., 2015).

We compared the clinicopathological findings and laboratory results of rangeliosis in domestic canines to an infection in a *C. thous*. Pathological and hematological findings in the crab-eating fox were similar to those found in infected dogs. The description of this case demonstrates that *C. thous* is not only a reservoir of the protozoan (Soares et al., 2014; Fredo et al., 2015), but can present with its associated disease as well.

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